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Abstract:

The ecology of Madagascar's grasslands is under-investigated and the dearth of ecological understanding of how disturbance by fire and grazing shapes these grasslands stems from a perception that disturbance shaped Malagasy grasslands only after human arrival. However, worldwide, fire and grazing shape tropical grasslands over ecological and evolutionary timescales, and it is curious Madagascar should be a global anomaly. We examined the functional and community ecology of Madagascar's grasslands across 71 communities in the Central Highlands. Combining multivariate abundance models of community composition and clustering of grass functional traits, we identified distinct grass assemblages each shaped by fire or grazing. The fire-maintained assemblage is primarily composed of tall caespitose species with narrow leaves and low bulk density. In contrast, the grazer-maintained assemblage is characterized by mat-forming, high bulk density grasses with wide leaves. Within each assemblage, levels of endemism, diversity and grass ages support these as ancient assemblages. Grazer-dependent grasses can only have co-evolved with a now-extinct megafauna. Ironically, the human introduction of cattle likely introduced a megafaunal substitute facilitating modern day persistence of a grazer-maintained grass assemblage in an otherwise defaunated landscape, where these landscapes now support the livelihoods of millions of people.

Key words: cattle, fire, grazing, grassland, functional traits, megafauna.

Introduction

The grasslands of Madagascar have long been considered degraded wastelands (e.g. Perrier de la Bâthie, 1921; Koechlin et al., 1974; Lowry et al., 1997). Consequently, little effort has been made to investigate their ecology, yet these grasslands cover over half the island (Moat and Smith, 2007). Recently, endemic grass lineages have been found to have evolved in Madagascar many millions of years before human arrival (Vorontsova et al., 2016; Hackel et al., 2018). It has been suggested that modern grasslands expanded significantly via people introducing cattle and bringing fire (Burney et al., 2003). Secondary grassy ecosystems, the result of forest degradation and agricultural conversion do exist across the island (Kull, 2004) but their distinction from ancient grasslands remains confusing. However, modern fire regimes in Malagasy grasslands have been identified where humans have limited influence, with fire return intervals of one to three years (Alvarado et al., 2018), similar to fire regimes of African grasslands with similar climates and where grasslands are considered ancient (Cerling et al., 1997; Jacobs et al., 1999; Stromberg, 2005, Edwards et al., 2010, Lehmann et al., 2011). Humans arrived around 10500 B.P. and anthropogenic landscape modification ca. 2300 BP lead to the megafaunal extinction alongside the introduction of cattle, where both overlapped by around 1500 years (Burney et al., 2004; Hansford et al., 2018; Douglass et al., 2019). Indeed, fire and grazing are likely to have been fundamental in both the evolution of these Malagasy grasslands and their modern dynamics. Despite being of fundamental relevance to supporting livelihoods, conservation and resolving contentions over ancient Malagasy ecosystems (e.g. Bond et al., 2008; Godfrey and Crowley, 2016), there has been sparse examination of the ecology of grasses (e.g. Rakotoarimanana and Grouzis, 2008).

In the past, a diverse vertebrate herbivore assemblage of now extinct primates, hippos, elephant birds and giant tortoises inhabited the island (Dewar, 1984) and that were suggested to have utilized grasslands (Burney et al., 2003). Hippos and giant tortoises are prime grazer candidates (Bond et al., 2008; Godfrey and Crowley, 2016) but carbon isotope data exist for only few specimens from the grassy centre of the island and evidence to support a grazer assemblage is limited (Godfrey and Crowley, 2016). Existing isotopic data show that hippos and tortoises consumed primarily C_3 plants with a variable C_4 plant component (Godfrey and Crowley, 2016) although emerging evidence supports a more mixed C_3-C_4 diet (Samonds et al., 2019). Understanding links between grasslands and the extinct fauna is crucial to determining the pre-settlement extent of the C_4- dominated grassy biomes.

Tropical grasslands the world over are structured by fire and grazing interacting with climate and soils (Bond, 2008; Lehmann et al., 2011). As top-down controls, fire and grazing transform organic materials to modify community structure and act as evolutionary agents (Bond and Keeley, 2005). However, each process has different requirements. Grazing mammals require nutritious nitrogen rich moist forage while fire consumes senesced carbon-rich plant material (Hempson et al., 2019). Thus, frequent fire versus frequent grazing leads to divergences in community composition (Trager et al., 2004, Hempson et al., 2019). Fire-associated grasses have traits promoting flammability and fire tolerance while grazing-lawn grasses have functional traits enabling proliferation under intense grazing but only where grazing is regular and concentrated. That is, the competitiveness and tolerance of grass life history strategies to each consumer-control initiates positive feedbacks between plant functional traits and consumer controls (Hempson et al., 2019).

The main argument for the anthropogenic assembly of Malagasy grasslands is low diversity (Perrier de la Bâthie, 1921; Lowry et al., 1997) and a lack of geographic structure (Koechlin, 1972). However, the diversity of the Malagasy grass flora is in line with most other islands of a similar size while endemicity is higher, at approximately ~40% (Vorontsova et al., 2016), and the geography of Malagasy grasslands has been little investigated (Koechlin, 1993). Given that similar expanses of grasslands occur in a similar range of rainfall across Africa, Australia, and the Americas where grasslands are recognised as natural and ancient (Cerling et al., 1997; Jacobs et al., 1999; Stromberg, 2005, Edwards et al., 2010; Lehmann et al., 2011), it is puzzling Madagascar should be an anomaly in biome distributions. On the African continent, compositional differentiation among grasslands can be linked to grazing and fire regimes that promote functionally divergent grassy ecosystems (e.g. Trager et al., 2004; Forrestel et al., 2015; Hempson et al., 2019). Here, we develop an overdue new understanding of the functional ecology and biogeography of grasslands across central Madagascar.

Materials and methods

Study sites

We sampled the grass flora at 71 sites across the central ecoregion of Madagascar among the regions of Ibity, Itremo, Isalo, Ankazobe and Antsirabe [Humbert, 1955; (Supplementary Figure 1)]. Data from 21 sites were from Solofondranohatra et al. (2018). The vegetation across the central ecoregion is predominantly extensive grassland and savanna woodland with some

closed forest (Moat and Smith, 2007). Mean annual rainfall ranges between 1200 mm and 1700 mm (Worldclim Global Climate Data version 2; Fick and Hijmans, 2017, see Supplementary Figure 2) with a 5 to 7-month dry season (Rajeriarison and Faramalala, 1999). Soils are primarily ferralitic on sandstone and basement gneiss (Moat and Smith, 2007).

Data collection

Grass species community composition

Grass species sampled at one site define a community in our analyses. In the field, community composition was quantified using the sampling method described in Vorontsova et al. (2016), to capture grass species diversity and relative frequency in a uniform vegetation area with a minimum area of 60 m x 60 m. All grass species within a centre circle plot of one metre diameter were recorded and, from this centre point, four 25-metre transects, each following a random direction (based on a compass bearing) from the point of origin were laid out. Along each transect, circular plots of one metre diameter were sampled at five metre intervals, representing grass species composition over 16.5 m^2. Species lists and their occurrences are presented in Supplementary Table 1.

Species rarity

Species were defined as rare based on two criteria: 1) the maximum frequency of a species within a community was less than five of 21 circular plots, and 2) the species occurred in five or fewer of the 71 grass communities assessed. Analyses involving grass functional traits were undertaken on species that were not rare. Based on this assessment, grass functional traits of 41 common grass species were collected. While a further 26 species were recorded, their functional traits were not assessed due to rarity.

Grass functional traits related to fire and grazing

Functional traits capture dimensions of life history strategies via quantifying morphology and architecture. We measured five grass functional traits related to flammability, palatability, and tolerance to fire and grazing. 1) Plant height, defined as leaf table height [the height measured and visually estimated to correspond to the c. 80th quantile of leaf biomass] has consequences

for light competition with taller grasses effective at competing for light (Dìaz et al., 2016), and flammability as taller grasses are generally high in biomass (Simpson et al., 2016). 2) Leaf thickness influences palatability with thick tough leaves less digestible (Wilson et al., 1983), flammability as leaves with higher C:N ratios are more flammable. 3) Ratio of leaf width to leaf length reflects leaf shape with wide short leaves preferred by grazers as palatable and long narrow leaves ignite easily and burn intensely (Schwilk, 2015). 4) Bulk density defined as mass per unit volume, relates to palatability and flammability. High bulk density grasses provide more forage per bite whereas low bulk density grasses provide aerated fuel beds (Hempson et al., 2019). 5) Architectural growth form of a grass can define the location of meristematic tissues to resist grazing and fire (Linder et al., 2018). Fuller details on functional traits and collection methods are described in Supplementary Table 2.

Environmental variables

Environmental data for Madagascar is of poor quality with few reliable weather stations, necessitating use of global and modelled products. We calculated four environmental variables to examine the geography of grass community. 1) Mean annual rainfall (MAP) was obtained from Worldclim Global Climate Data (Fick and Hijmans, 2017) as proxy for productivity (Huxman et al., 2004). 2) Percent sand in the top 10 cm of soil ('sand percent') was obtained from Harmonised World Soils Database (FAO 2009) that reflects soil water holding capacity where sandy soils have low water holding capacity, thus partly capturing patterns of landscape water availability. 3) The presence/ absence of fire was scored for each site based on interviews with local communities and land managers. 4) Distance to road was used as a proxy for grazing pressure and quantified using the national roads layer for Madagascar (Foiben-Taosarintanin'i Madagasikara, 1997) with three levels of road (tarred, untarred, track). Cattle are the dominant grazer across Madagascar, and cattle are associated with human communities that are largely associated with roads. Some main roads through the Central Highlands also follow river valleys and can also reflect landscape water availability and soil properties which are also important to shaping potential cattle densities. Values of these environmental variables across our 71 studied sites are given in Supplementary Figure 2.

Analyses

Modelling grass species assemblages

Generalized latent variable models were used to determine whether distinct grass assemblages could be identified across sites based on patterns of species co-occurrences (Skrondal and Rabe-Hesketh 2004) across 71 communities. Rare species as defined above were omitted from the analysis because they typically contribute little interpretive value while adding noise to the statistical solution (Gauch, 1982). Accordingly, 41 of 67 species were used in our assemblage analyses.

Relative species frequencies of each species in each community was the response variable. Candidate models comprised the full set of additive permutations of four environmental variables in addition to a single unobserved predictor (latent variable). All environmental variables were scaled prior to analysis, with MAP and distance to road being base-10 log transformed to meet model assumptions. Models were fitted in R (R version 3.0.2; R Core Team, 2013) using the gllvm package (Niku et al., 2018), specifying a negative binomial error distribution and accounting for spatial autocorrelation by including site latitude and longitude as variables.

Identifying grass species assemblages and environmental associations

Model comparisons were based on the Akaike Information Criterion (AIC; Akaike, 1981, 1983). Using the most supported model, species assemblages were identified based on the matrix of residual correlations along with histograms of residual correlations for each species to identify natural breaks in residual correlation values (Supplementary Figure 3). Residual correlation values range from -1 to +1. Based on the histograms, species grouped naturally into two assemblages where values were: 1) > 0.1 and 2) <-0.1. Species with residual correlations ranging from -0.1 to +0.1 represent a lack of any association and species were not classified into either assemblage as they may be equally likely and unlikely to co-occur.

Rare species not incorporated into the gllvm analyses were assigned a post-hoc assemblage group, made possible by the very strong species co-occurrence patterns. To classify these 21 species, each community was assigned an assemblage group based on the dominant proportion of species in each assemblage group. Assemblage assignments for the 21 rare species enabled us to undertake analyses of phylogenetic conservatism described later. Finally, the relationship between each environmental correlate and species assemblages was assessed by plotting model coefficients of environmental correlates values for each assemblage group using boxplots.

Identifying grass functional types

We sought to identify syndromes of functional traits that represent functionally similar species. These functional groups could then be cross-referenced with assemblage groups. Functionally similar species were identified using hierarchical clustering on principal components (HCPC) of the five functional traits described above for the 41 common grass species. Clustering used the Ward method based on Euclidian distance. The final number of clusters was determined using the sum of within-cluster inertia (Husson et al., 2018) where the final number of clusters corresponded with the highest relative loss of inertia. Functional trait values were then plotted for each cluster using violin plots and clusters were compared using analysis of variance (ANOVA).

Species evolutionary history

To explore phylogenetic patterns of grass species relative to assemblage groups and functional traits, we extracted the Bayesian time-calibrated phylogenetic tree of the species from a large analysis of Malagasy grasses (Hackel et al., 2018). *Digitaria thouaresiana*, *Eragrostis atrovirens* and *Schizachyrium exile* had no DNA available and were not included. *Paspalum scrobiculatum* was replaced by the only species within the Paspaleae tribe (*Hildaea pallens*) in Hackel et al. (2018), and *Axonopus compressus* was inserted based on its estimated divergence from *Paspalum* in Christin et al. (2014).

Three species level attributes were plotted against the phylogenetic tree of 64 species, these were: 1) Assemblage group; 2) Functional group, and 3) Endemicity [obtained from the Global Biodiversity Information Facility (GBIF)].

Four analyses were then undertaken to test: 1) Differences in species richness (Whittaker, 1972) and phylogenetic diversity (PD; Faith, 1992) between the two assemblage groups; 2) Differences in endemicity between the two assemblage groups; 3) Distribution of species functional traits along the phylogeny between the two assemblage groups; and 4) Phylogenetic conservatism of functional traits. Each test respectively used: 1) A generalized linear model (GLM) with a Poisson distribution and log link function; 2) A two-proportions z-test; 3) A phylogenetic ANOVA using "phytools" package (Revell, 2012); and, 4) An estimation of Blomberg's K (Blomberg et al., 2003) with the "phylosig" function using 999 numbers of tree shuffling randomization.

Results

Assemblage groups

Residual correlations very clearly identified two species groups (Figure 1). The most supported model generating these groups included mean annual precipitation, distance to road and presence/absence of fire as environmental correlates (AIC = 4904.07, ΔAIC to second-best model = 2.18, Figure 1, Supplementary Table 3). Group "1" (top of the correlation matrix) composed of species highly likely to co-occur with significant positive correlations (Figure 1). Species from Group 1 were highly unlikely to co-occur with any species in Group "2", all of which are characterized by significant negative correlations (Figure 1). Six species had residual correlation values ranging from -0.1 to +0.1 (Figure 1, Supplementary Figure 3) and were not classified into either assemblage. Assemblage groups corresponding to each analysed species are presented in Supplementary Table 1.

Linking Assemblage groups with environment

Mean annual precipitation and presence of fire had largely negative associations with Assemblage group 1, and positive associations with Assemblage group 2 (Figure 2). Two species had very large coefficients related to rainfall. These were: *Brachiaria subrostrata* and *Pennisetum pseudotriticoides* with coefficients respectively of -1030 and 690. In contrast, *Brachiaria subrostrata* had a strongly negative coefficient related to fire presence/absence (-148). Extreme coefficients relate to the absence of these species from many communities with the model for mean frequency appropriately fitted on a log scale. Distance to road has variable relationship with Assemblage 1 and mainly positive relationships with Assemblage 2 (Figure 2).

Syndromes of grass functional traits

Hierarchical clustering identified three functional groups of species associated with grazing and fire alongside an intermediate group (harbouring traits between the two groups) (Figure 3A). Significant differences were found between all numerical mean trait values of the three groups ($P < 0.001$, Figure 3B). The grazing group of fourteen species, more than half of which

are mat forming (57.1% of the group) and with all sampled mat-forming species within this group are short grasses with high bulk densities, and short wide thin leaves. Leaf width to length ratio and bulk density were similar between grazing and intermediate groups (all P > 0.05) but far higher than the fire group (all $P < 0.001$). The fire group comprises 23 species, all of which are tall caespitose grasses with thicker leaves, low bulk density and low leaf width to length ratios compared to the grazing group (all $P < 0.001$). Species in the intermediate group have similar bulk densities as species in the fire group ($P > 0.05$).

Linking assemblage and functional groups

We found high correspondence between the assemblage and functional analyses (Table 1). Thirteen of 14 species in the grazing functional group (92.85 %) are found in Assemblage 1. Of the 22 species within Assemblage 1 (59.1 %) were clustered in grazing group. In contrast, Assemblage 2 is strongly associated with the fire functional group with 12 of the 13 species in Assemblage 2 found in the fire-grass functional group. Chi-square test result showed that functional and residual groups have a significant relationship ($P = 0.001$). Among the 41 species for which there are functional data, there are 11 endemic species, of which five each are respectively found in Assemblage 1 and 2. Four endemic species are found in the grazing-adapted functional group, seven in the fire-adapted functional group and none in the intermediate group. Based on the evidence, Assemblage 1 represents a suite of grazer-maintained communities while Assemblage 2 represents a suite of fire-maintained communities.

Species evolutionary history

The two assemblages are phylogenetically over-dispersed (Figure 4). Of the 67 sampled species, 31.4 % are endemic. Twelve endemic species are associated with the fire-maintained assemblage and eight with the grazing-maintained assemblage. One endemic species (*Andropogon trichozygus*) has residual correlation values ranging from -0.1 to +0.1 and is among the group of species not classified into either assemblage. There are no significant differences between the proportion of endemics of the two assemblages (P> 0.05) while accounting for phylogeny. However, a phylogenetic ANOVA found that variances within assemblages are associated with grass leaf table height (P = 0.008, F = 4.26) and bulk density

(P = 0.04, F = 2.59) but not leaf size or thickness. The species richness is similar between the two assemblage groups, and phylogenetic diversity within grazing-maintained assemblage is significantly higher than fire-maintained assemblage (Supplementary Figure 4). No significant phylogenetic signal was found in any of the functional traits, indicating that these are evolutionarily labile (all P > 0.05 for the four numerical traits).

Discussion

In Madagascar, grasslands are far from a homogenous landscape but, much like in continental Africa, are shaped by the contrasting processes of fire and grazing that promote differentiation in community composition where constituent species have diverging syndromes of functional traits. In our research, Malagasy grass communities shaped by grazing and fire each have ~30-40% endemism (Table 1, Figure 4). These endemic grazer and fire specific species pre-date human arrival [(ca. 10500 B.P.), Hansford et al., 2018; Douglass et al., 2019] by millions of years, with a divergence age range of 1-7 million years (Hackel et al., 2018) suggesting that grazing animals and fire shaped community assembly in a functionally comparable way to grassland systems in Africa well before human arrival.

Malagasy grazing lawn communities (Assemblage 1 and grazing functional group; Figure 1, 3 and 5) were characterized by short, mat-forming, high bulk density grasses with short wide thin leaves. Grazing lawns can only spread and persist under consistent concentrated grazing that limits light competition from other grass species (McNaughton 1988, Hempson et al., 2015) but also requires that grass species keep meristematic tissue at or below the soil surface, and thus inaccessible to grazers, to tolerate such consistent grazing. Fire driven communities (Assemblage 2 and fire functional; Figure 1, 3 and 5) are characterized by similar species richness and lower phylogenetic diversity relative to grazing communities (Supplementary Figure 4) with tall caespitose grasses with low bulk density and longer, narrower and thicker leaves. Tall grasses, usually with a high aboveground biomass quantity and low bulk density (i.e. sparse architecture) are highly flammable and promote fire (Simpson et al., 2016). The presence of numerous endemic grasses within the system strengthens the evidence that some extent of fire-maintained grasslands are a natural and ancient part of the region.

Despite the congruence identified between assemblage and functional groups, a small suite of species did not match between analyses. We interpret these species as being potentially able to persist in communities shaped either by fire or grazing through tolerating both consumers to

some degree. These species, such as *Hyparrhenia rufa*, *Heteropogon contortus and Sporobolus pyramidalis* also have pan-African or even cosmopolitan range sizes as would be expected if a species can tolerate a wide range of disturbance conditions (Archibald et al., 2019). In our dataset, these species were functionally clustered within the fire-grasses, but possibly as a product of traits being sampled where species were first encountered in our surveys, i.e., in frequently burnt communities, while these species were also found elsewhere.

Madagascar's now extinct megafauna, including hippos, giant tortoises, elephant birds and giant lemurs survived well into the Holocene (Burney et al., 2003, Goodman and Jungers, 2014), and their extirpation ca 1200 cal B.P. was well after anthropogenic landscape modification is noted in the palaeo-record (Hansford et al., 2018; Douglass et al., 2019). Malagasy hippos, members of the derived genus *Hippopotamus* arrived in Madagascar in the Quaternary (Simpson, 1940; Mahé, 1972). Recent isotope data suggest that hippos in central Madagascar consumed a mixed diet of C_3 and C_4 plants in an open ecosystem (Samonds et al., 2019) although previous isotope data suggested a primarily C_3 diet where the majority of grasses in the Central Highlands are C_4 (Godfrey and Crowley, 2016). In Africa, hippos are short-grass grazing specialists that play a crucial role in initiating and maintaining grazing lawns in areas of high rainfall (Verweij et al., 2006; Hempson et al., 2015) similar in rainfall to our study sites. Although hippos isotopic values in Africa are higher [Cerling et al., 2008 ($\delta^{13}C$ = -3.6‰), Boisserie et al., 2005 ($\delta^{13}C$ = -3.5‰)] compared to Malagasy hippos [Samonds et al., 2019 ($\delta^{13}C$ = -15.9‰)], it does suggest a mixed C_3 and C_4 diet. Samonds et al. (2019) suggest that Malagasy hippos may be ecologically comparable to the African pygmy hippo, *Choeropsis liberiensis*. A mixed diet would also be supported by the abundance of C_3 forbs common to grazing lawns that can be highly palatable (O'Connor, 1991). In Madagascar, tortoises were also known to consume some proportion of C_4 and/ or CAM plant material (Burleigh and Arnold, 1986, Godfrey and Crowley, 2016) and both C_3 and C_4 plants on the Mascarene islands (van der Sluis et al., 2014). A high density of tortoise can effectively keep grass short and unable to carry fire (e.g. Burney et al., 2015). It should be noted that isotope records in Madagascar are not complete in the Central Highlands possibly as preservational environments are limited and areas of possible preservation have long been suited to agriculture. We suggest, the ecology of the grasses examined here demonstrates that in the early Pliocene, megagrazers most likely hippos and giant tortoises were instrumental in the evolution and assembly of the Malagasy Central Highlands obligate grazing lawn flora (Figure 4), and that cross-disciplinary efforts to reconcile palaeo and ecological data are much needed.

The geography of grazing lawns and fire grasslands is not random but related to rainfall, distance to roads and the presence of fire (Figure 2) that also represent a legacy of human colonisation and patterns of modern land use. Sites with higher rainfall were more likely to have fire, while sites with lower rainfall were more likely associated with grazing. Across the rainfall gradient sites located near roads are more likely subject to intensive concentrated grazing. Undoubtedly, the modern dynamics of grazing lawns in Madagascar are shaped by cattle raised close to roads (or waterways), where people live and can manage them relatively easily in terms of forage and safety. But the associations of species dependent upon grazing are likely ancient, evidenced by the species diversity and endemicity. Cattle, hippos and grazing tortoises share key functional similarities, they prefer highly palatable grasses with high bulk density to maximise intake of nutritious food per bite. McCauley et al. (2018) showed that a mixture of herbivores (including cattle and hippos) and removal of hippos on grazing lawns in East Africa similarly impacted grassland diversity and structure, suggesting some functional equivalence between hippos and livestock. The replacement of one grazer with another is unlikely to have substantially reshaped diversity where an obligate grazing flora already existed. While grazing lawns in Africa are maintained by a diversity of wild mammal grazers, cattle increasingly maintain grazing lawns due to the vast and extensive displacement of native grazers with livestock. In Africa, grazing lawns also support a diversity of grass species (Hempson et al., 2015) with diversity in Malagasy grazing lawns similar or greater (O'Connor, 2001; O'Connor, 2005). The current decline and extinction of African megafauna may well be an analogy of the historic megafaunal extinctions in Madagascar, where productive landscapes now used for cattle rearing are fundamentally underpinned by an ancient obligate grazing flora, a product of millions of years of grazer and grass co-evolution.

Examination of the impacts of megafaunal extinction generally focuses on woody plants rather than grasses. While grasses can be long lived, it would be possible for grazing grasses in particular to be rapidly lost from ecosystems when over-topped by taller grasses or woody plants. Indeed, the temporal overlap between the megafaunal extinction and arrival of cattle may have been the salvation of the Malagasy grazing grass flora while also facilitating human colonisation of the island. It will be crucial to understand the impacts of environmental change on these ancient grass assemblages with droughts increasing in frequency and severity. However, also much needed is identification of the limits of ancient and modern grassland ecosystems requiring collaboration across disciplines. In Madagascar, grasslands are dismissed as wastelands in need of forest restoration. Hence, grasslands are now the subject of extensive

tree planting programs, adopted as environmental policy for forest restoration, carbon sequestration and fuelwood production. The most commonly planted trees are exotic *Eucalyptus*, *Acacia* and *Pinus* species, species known as invasive elsewhere in the world. Food security in Madagascar is highly precarious and where agriculture in the Central Highlands is dependent on abundant stream flow for rice production. If grasslands are an extensive ancient component of these Central Highlands landscapes, which is likely given the patterns of diversity, geography and endemism observed here, not only is planting of exotic trees species damaging, but at scale will likely reduce stream flow (Jackson et al., 2005) with unforeseen environmental consequences in a changing climate. Malagasy grasslands require new science to help delimit pre-human versus modern limits linked to the assemblages identified here. There is a clear need for science to engage with regions hitherto dismissed as being of no value for the sake of future conservation, land management and livelihoods.

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Figures

Figure 1. Residual correlation values between pairs of 41 grass species derived from 71 grasslands. Values indicate the likelihood of pairwise species co-occurrence that identified two major grassland assemblages: “Group 1” (top of the matrix) and “Group 2” (bottom right of the matrix). Group 1 species are highly likely to co-occur but not with species in Group 2. Significant ($P < 0.05$) positive correlations are represented by blue cells, and significant negative associations correspond to red cells. Non-significant associations are blank. Correlation values are estimated from a generalized linear latent variable model incorporating mean annual precipitation, presence/absence of fire, distance to road and a single latent variable.

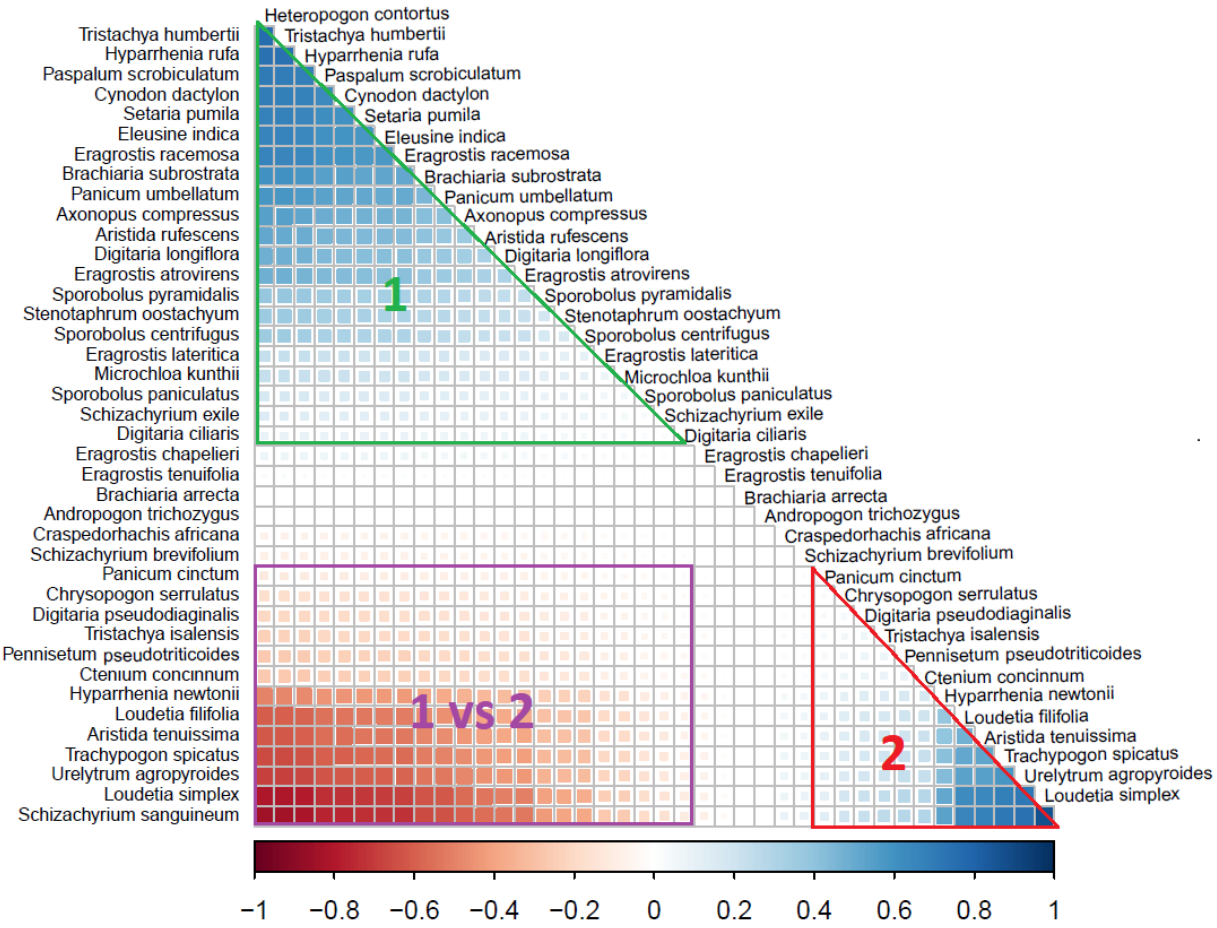


Figure 2. Model coefficients of environmental correlates compared between grass assemblage group. Coefficients are related to (A) mean annual precipitation (mm per year), (B) distance to road (m) and (C) presence or absence of fire.

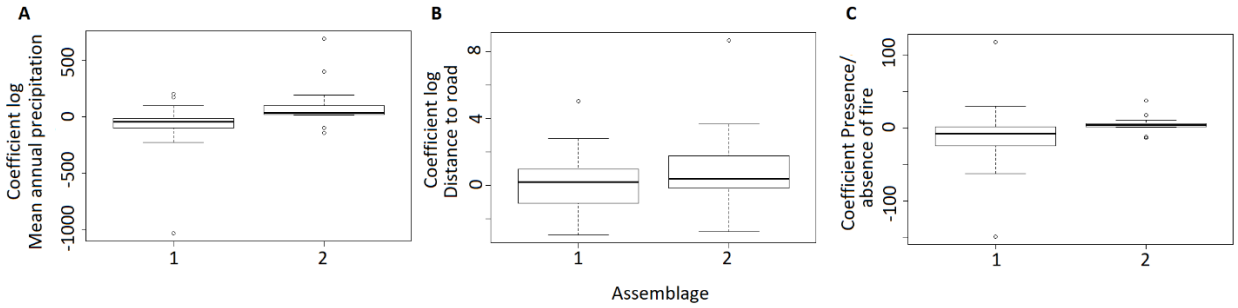
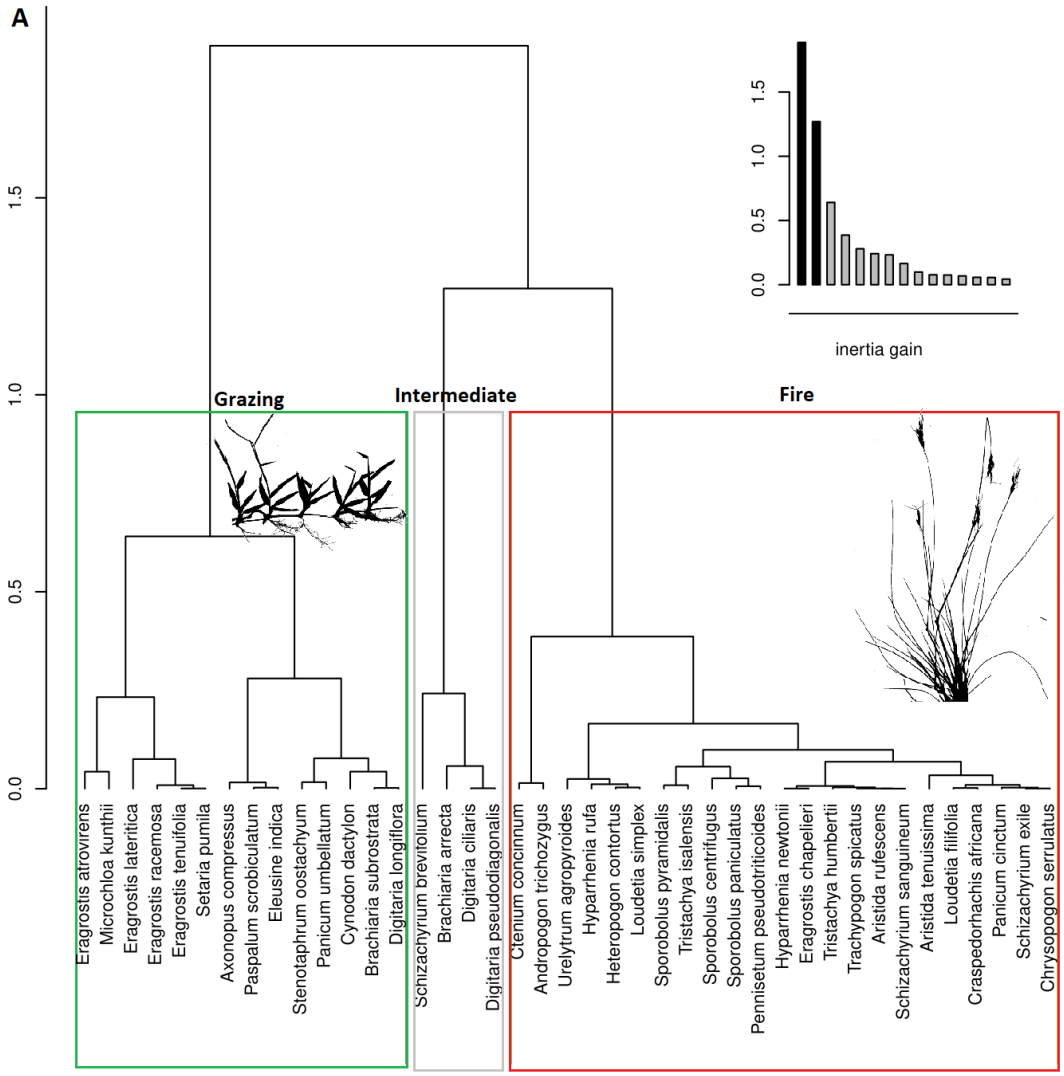


Figure 3. Three cluster of grass species representing significant differences among groups in three functional traits. (A) Dendrogram produced via hierarchical clustering on principal component (HCPC) of 41 grass species based on growth form, leaf width to length ratio, leaf thickness, bulk density and leaf table height. Three functional groups are supported and interpreted as related to: grazing; intermediate (traits enabling tolerance of some level of both grazing and fire); and fire. Black silhouettes represent typical grazing (*Cynodon dactylon*) and fire (*Loudetia filifolia*) grass morphologies. (B) Violin plots of four functional traits per functional group from the HCPC dendrogram. There are significant differences in all the traits between the functional clusters ($P < 0.001$ for each).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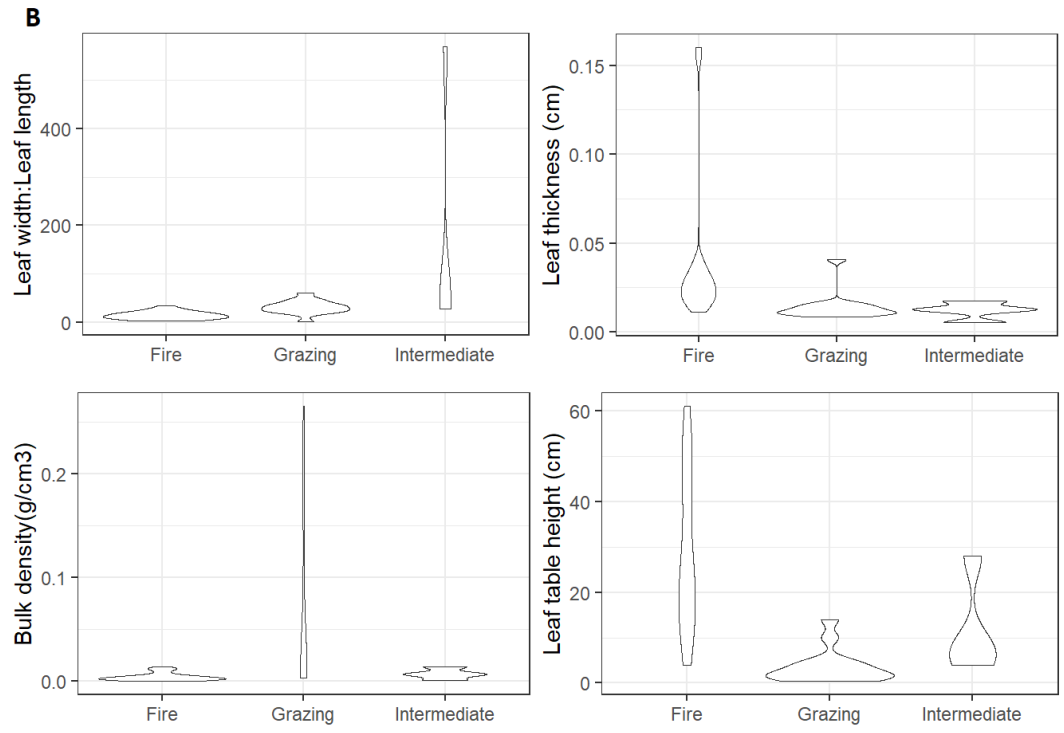
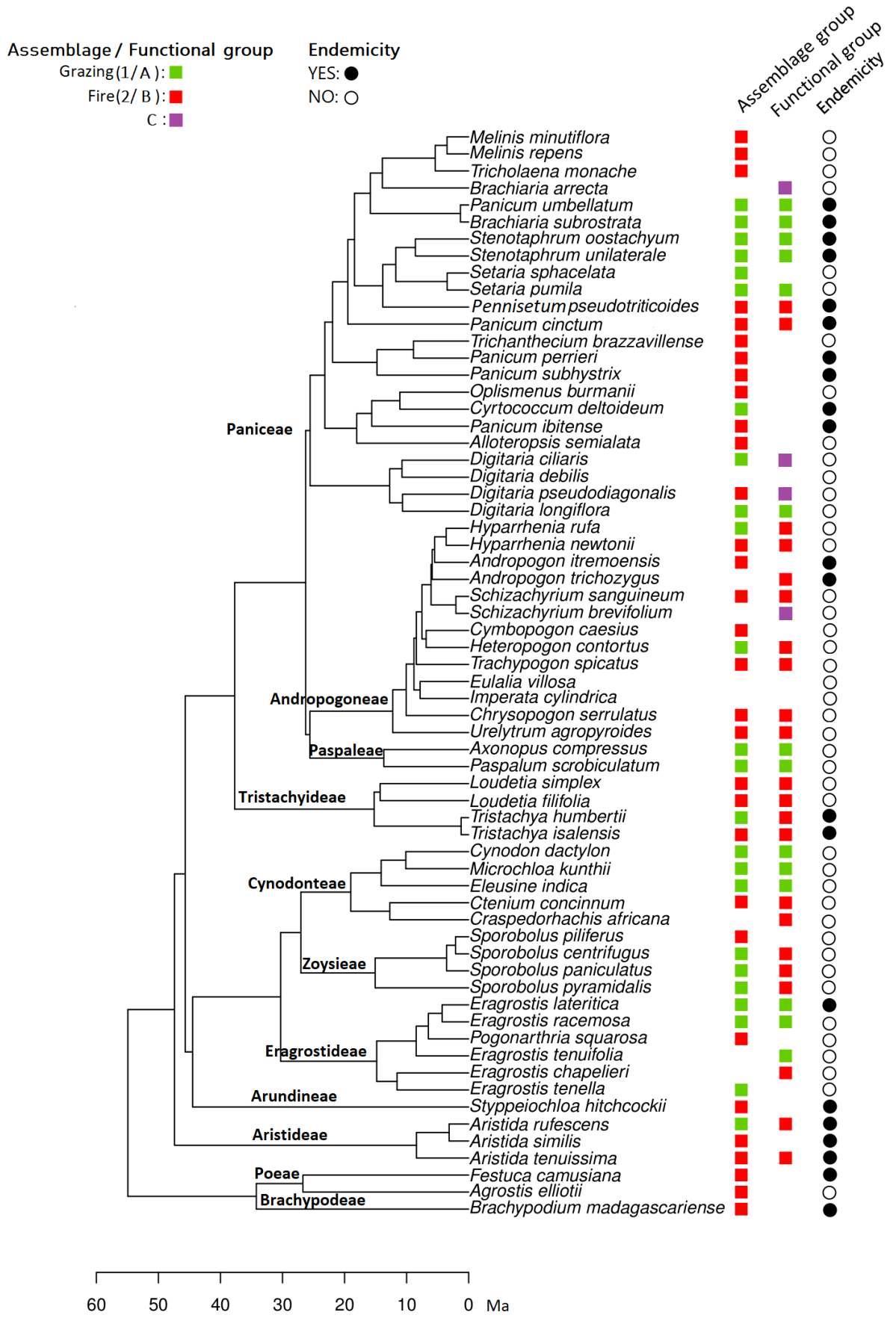
Axonopus compressus
Paspalum scrobiculatum
Eleusine indica
Stenotaphrum oostachyum
Panicum umbellatum
Cynodon dactylon
Brachiaria subrostrata
Digitaria longiflora
Schizachyrium brevifolium
Brachiaria arrecta
Digitaria ciliaris
Digitaria pseudodiagonalis
Ctenium concinnum
Andropogon trichozygus
Urelytrum agropyroides
Hyparrhenia rufa
Heteropogon contortus
Loudetia simplex
Sporobolus pyramidalis
Tristachya isalensis
Sporobolus centrifugus
Sporobolus paniculatus
Pennisetum pseudotriticoides
Hyparrhenia newtonii
Eragrostis chapelieri
Tristachya humbertii
Trachypogon spicatus
Aristida rufescens
Schizachyrium sanguineum
Aristida tenuissima
Loudetia filifolia
Craspedorhachis africana
Panicum cinctum
Schizachyrium exile
Chrysopogon serrulatus


B
Leaf width:Leaf length
400
200
0
Fire
Grazing
Intermediate
Leaf thickness (cm)
0.15
0.10
0.05
0.00
Bulk density(g/cm3)
0.2
0.1
0.0
Leaf table height (cm)
60
40
20
0

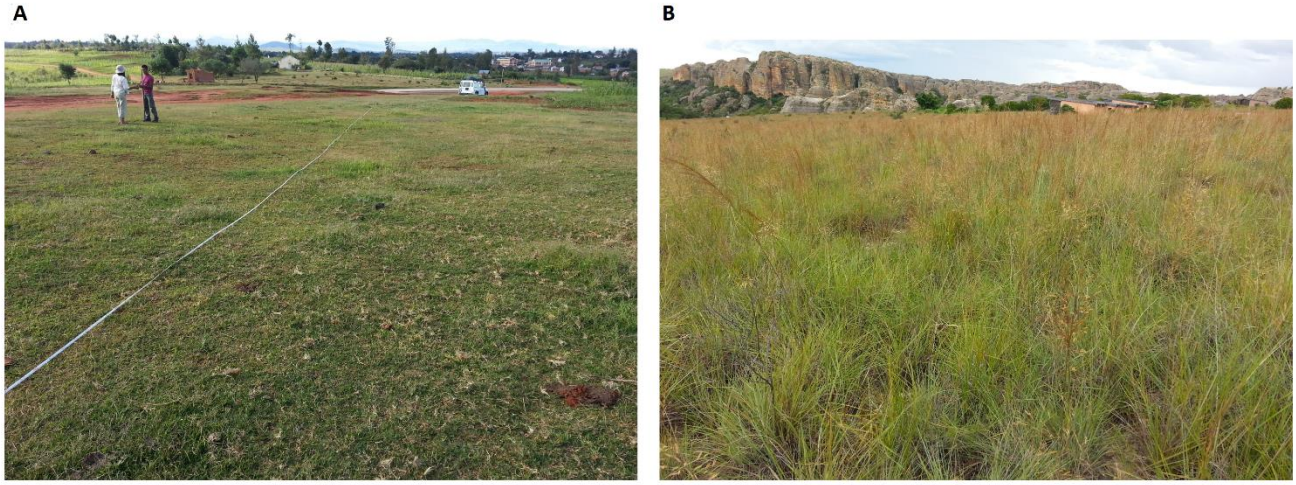
430

Figure 4. Phylogenetic tree of all 64 grass species mapped to: a) Two assemblage groups (for all species except those that could not be attributed to either Assemblage); b) Three functional groups of the 39 common species (*Eragrostis atrovirens* and *Schizachyrium exile* are not included due to lack of available sequences); and, c) Species endemicity.


Assemblage / Functional group
Grazing (1/A):
Fire (2/B):
C:
Endemicity
YES:
NO:
Assemblage group
Functional group
Endemicity
Melinis minutiflora
Melinis repens
Tricholaena monache
Brachiaria arrecta
Panicum umbellatum
Brachiaria subrostrata
Stenotaphrum oostachyum
Stenotaphrum unilaterale
Setaria sphacelata
Setaria pumila
Pennisetum pseudotriticoides
Panicum cinctum
Trichanthecium brazzavillense
Panicum perrieri
Panicum subhystrix
Oplismenus burmanii
Cyrtococcum deltoideum
Panicum ibitense
Alloteropsis semialata
Digitaria ciliaris
Digitaria debilis
Digitaria pseudodiagonalis
Digitaria longiflora
Hyparrhenia rufa
Hyparrhenia newtonii
Andropogon itremoensis
Andropogon trichozygus
Schizachyrium sanguineum
Schizachyrium brevifolium
Cymbopogon caesius
Heteropogon contortus
Trachypogon spicatus
Eulalia villosa
Imperata cylindrica
Chrysopogon serrulatus
Urelytrum agropyroides
Axonopus compressus
Paspalum scrobiculatum
Loudetia simplex
Loudetia filifolia
Tristachya humbertii
Tristachya isalensis
Cynodon dactylon
Microchloa kunthii
Eleusine indica
Ctenium concinnum
Craspedorhachis africana
Sporobolus piliferus
Sporobolus centrifugus
Sporobolus paniculatus
Sporobolus pyramidalis
Eragrostis lateritica
Eragrostis racemosa
Pogonarthria squarosa
Eragrostis tenuifolia
Eragrostis chapelieri
Eragrostis tenella
Styppeiochloa hitchcockii
Aristida rufescens
Aristida similis
Aristida tenuissima
Festuca camusiana
Agrostis elliotii
Brachypodium madagascariense
Paniceae
Andropogoneae
Paspaleae
Tristachyideae
Cynodonteae
Zoysieae
Eragrostideae
Arundineae
Aristideae
Poeae
Brachypodeae
60
50
40
30
20
10
0
Ma

435

Figure 5. Examples of typical grasslands in the Madagascar Central Highlands. (A) a grazing lawn in Ibity, containing 18 species and dominated by *Cynodon dactylon* (NE), *Panicum umbellatum* (E) and *Digitaria longiflora* (NE); (B) Fire-maintained grassland in Isalo containing eight species and dominated by *Loudetia simplex* (NE) and *Loudetia filifolia* (E). NE = Not endemic, a grass species with a distribution that spans Africa and Madagascar. E = endemic, a grass species restricted to Madagascar and Mascarene Islands.



443 **Tables.**

444 **Table 1.** Number of grass species in assemblage and functional groups. Number of endemics
445 per group are given in brackets.

	Assemblage group 1 (**Grazing**)	Assemblage group 2 (**Fire**)	Total species per functional group (including species which were not part of either assemblage groups)
Grazing group	13	0	14 (4 endemics)
Intermediate group	1	1	4 (0 endemic)
Fire group	8	12	23 (7 endemics)
Total per assemblage group	22 (5 endemics)	13 (5 endemics)	

446

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Supplementary Materials

Title: Fire and grazing determined grasslands of central Madagascar represent ancient assemblages.

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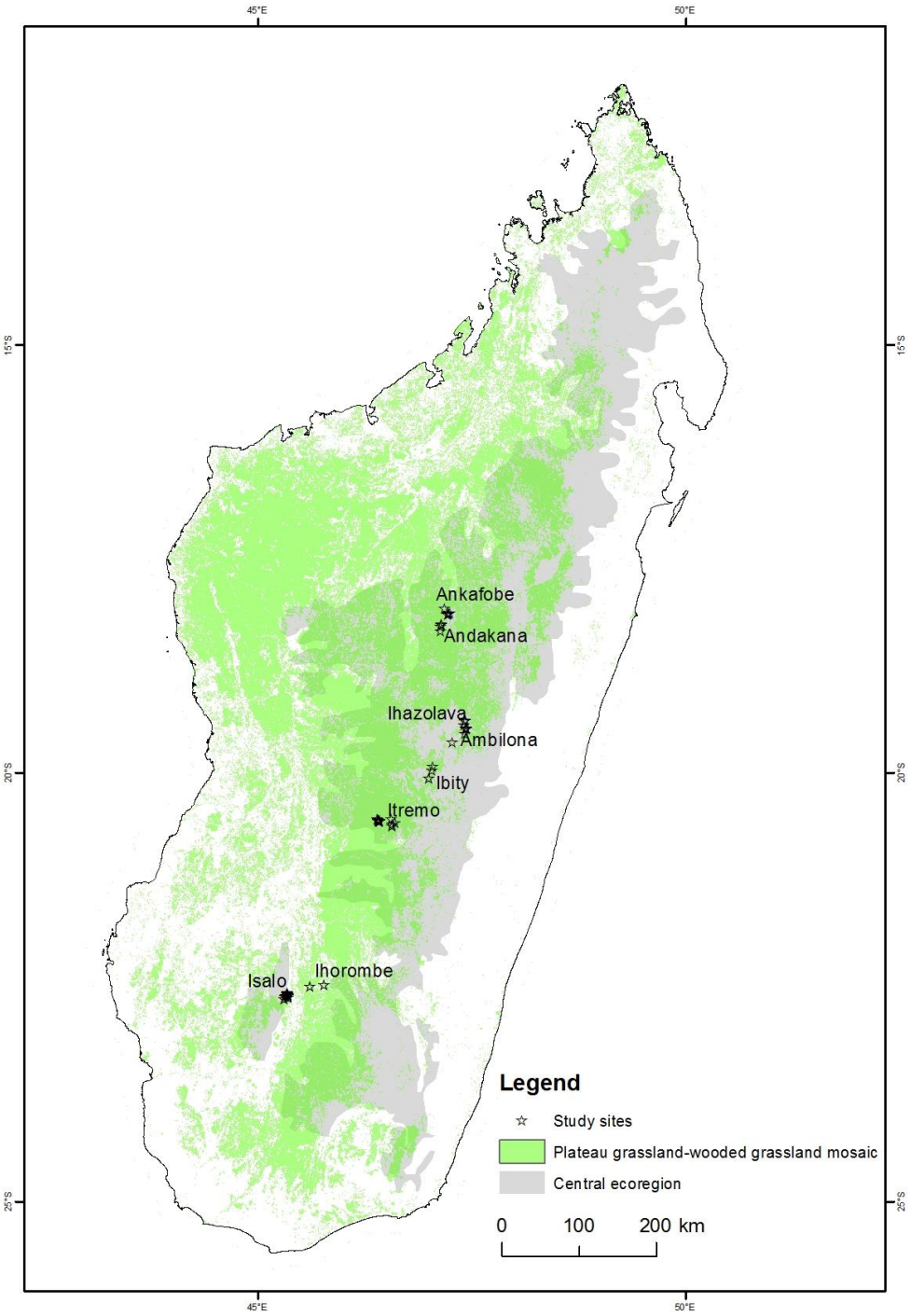
Supplementary Figures 1 – 4

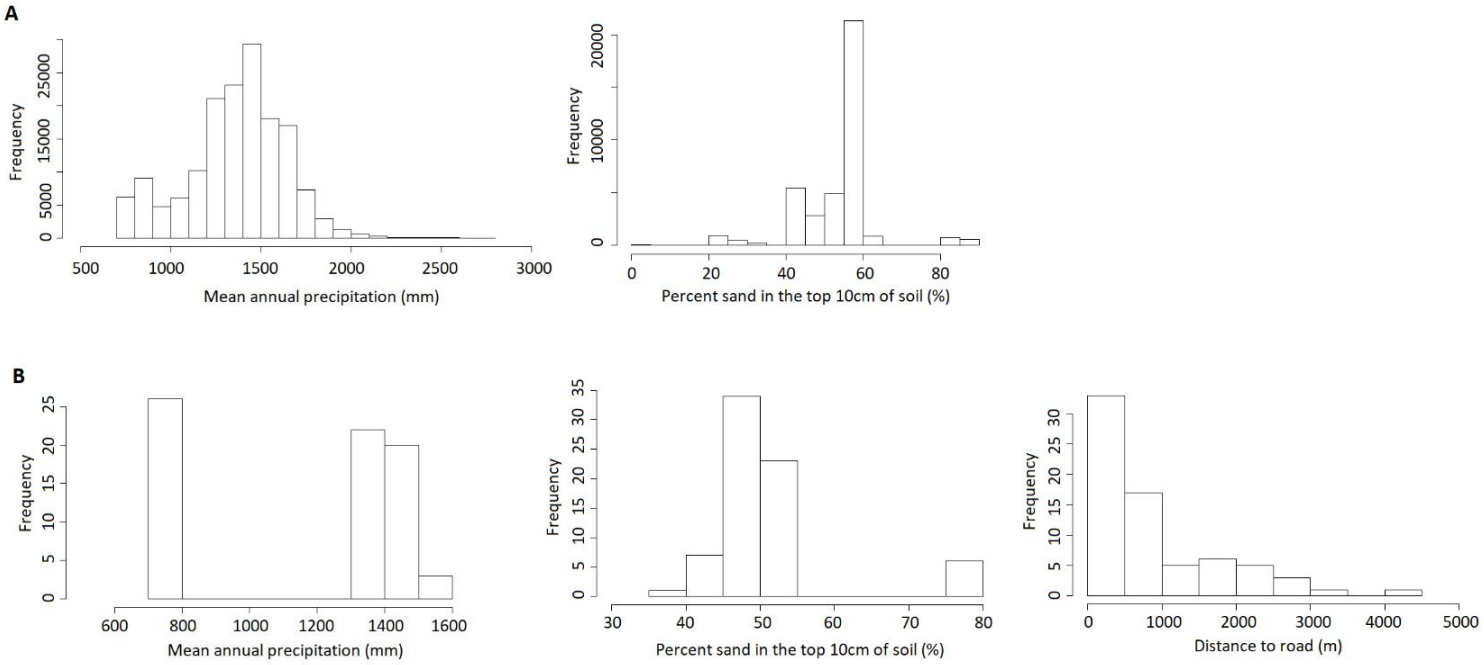
Supplementary Tables 1 – 3

References

Supplementary Figure 1: *Map of Madagascar depicting limits of grasslands*. The central ecoregion as per Humbert (1955) is shaded grey. Plateau grassland- wooded grassland mosaic distribution as per Moat and Smith (2007) is shaded in green. Locations of study sites are shown as are names of regions where sampling was undertaken.



Supplementary Figure 2: *Environmental variables distribution* (A) Histograms of mean annual precipitation (Bio_12, Worldclim Global Climate Data version) and percent sand in the top 10 cm soil (Harmonised World Soils Database) across the central ecoregion as mapped by Humbert (1955). (B) Across the 71 study sites, histograms of mean annual precipitation, percent sand in the top 10 cm soil, and distance to road.



Supplementary Table 1: *Table of all grass species encountered.* Table describes: 1) endemicity; 2) number of sites where species were found; 3) maximum number of occurrences per site (out of a maximum of 21); 4) rarity as defined and described in the methods of the main text; and, 5) assemblage group (1 or 2). Assemblage groups are based on residual correlations values between pairs of species as a product of the generalized linear latent variable model described in the main methods. The analysis used only 41 common species and post-hoc assemblage group were assigned to the rare species.

Genera	**Species**	**Endemic**	**Number of sites of occurrence (out of 71)**	**Maximum number of occurrence per site (out of 21)**	**Rare**	**Assemblage group**
Agrostis	*elliotii*	yes	1	1	yes	2
Alloteropsis	*semialata*	no	4	5	yes	2
Andropogon	*itremoensis*	yes	1	4	yes	2
Andropogon	*trichozygus*	yes	1	23	no	NA
Aristida	*rufescens*	no	12	20	no	1
Aristida	*similis*	yes	2	5	yes	2
Aristida	*tenuissima*	yes	21	24	no	2
Axonopus	*compressus*	no	4	7	no	1
Brachiaria	*arrecta*	no	1	17	no	NA
Brachiaria	*subrostrata*	yes	3	14	no	1
Brachypodium	*madagascariense*	yes	1	1	yes	2
Chrysopogon	*serrulatus*	no	11	20	no	2

Craspedorhachis	*africana*	no	18	15	no	NA
Ctenium	*concinnum*	no	5	16	no	2
Cymbopogon	*caesius*	no	4	5	yes	2
Cynodon	*dactylon*	no	12	20	no	1
Cyrtococcum	*deltoideum*	yes	1	1	yes	1
Digitaria	*ciliaris*	no	4	7	no	1
Digitaria	*debilis*	no	5	5	yes	NA
Digitaria	*longiflora*	no	25	21	no	1
Digitaria	*pseudodiaginalis*	no	6	6	no	2
Digitaria	*thouaresiana*	no	1	2	yes	NA
Eleusine	*indica*	no	9	18	no	1
Eragrostis	*atrovirens*	no	6	19	no	1
Eragrostis	*chapelieri*	no	1	6	no	NA
Eragrostis	*lateritica*	yes	17	15	no	1
Eragrostis	*racemosa*	no	6	13	no	1
Eragrostis	*tenella*	no	2	5	yes	1
Eragrostis	*tenuifolia*	no	4	6	no	NA
Eulalia	*villosa*	no	1	4	yes	NA
Festuca	*camusiana*	yes	1	1	yes	2
Heteropogon	*contortus*	no	9	21	no	1
Hyparrhenia	*newtonii*	no	19	15	no	2

Hyparrhenia	*rufa*	no	18	21	no	1
Imperata	*cylindrica*	no	5	5	yes	NA
Loudetia	*filifolia*	no	11	21	no	2
Loudetia	*simplex*	no	58	25	no	2
Melinis	*minutiflora*	no	5	3	yes	2
Melinis	*repens*	no	4	5	yes	2
Microchloa	*kunthii*	no	7	8	no	1
Oplismenus	*burmanii*	no	2	1	yes	2
Panicum	*cinctum*	yes	12	13	no	2
Panicum	*ibitense*	yes	4	5	yes	2
Panicum	*perrieri*	yes	3	2	yes	2
Panicum	*subhystrix*	yes	5	5	yes	2
Panicum	*umbellatum*	yes	23	21	no	1
Paspalum	*scrobiculatum*	no	16	18	no	1
Pennisetum	*pseudotriticoides*	yes	5	13	no	2
Pogonarthria	*squarosa*	no	2	2	yes	2
Schizachyrium	*brevifolium*	no	3	16	no	NA
Schizachyrium	*exile*	no	7	12	no	1
Schizachyrium	*sanguineum*	no	49	22	no	2
Setaria	*pumila*	no	14	20	no	1
Setaria	*sphacelata*	no	2	2	yes	1

Sporobolus	*centrifugus*	no	21	18	no	1
Sporobolus	*paniculatus*	no	3	7	no	1
Sporobolus	*piliferus*	no	1	1	yes	2
Sporobolus	*pyramidalis*	no	5	20	no	1
Stenotaphrum	*oostachyum*	yes	6	15	no	1
Stenotaphrum	*unilaterale*	yes	1	4	yes	1
Styppeiochloa	*hitchcockii*	yes	1	1	yes	2
Trachypogon	*spicatus*	no	45	25	no	2
Tricanthecium	*brazzavillense*	no	2	5	yes	2
Tricholaena	*monache*	no	3	5	yes	2
Tristachya	*humbertii*	yes	4	17	no	1
Tristachya	*isalensis*	yes	5	9	no	2
Urelytrum	*agropyroides*	no	6	19	no	2

Supplementary Table 2: *Description of five measured traits alongside collection method, related function and literature references.* The five traits are: 1) leaf table height (H_{LT}, cm); 2) leaf thickness (LT, cm); 3) leaf size: leaf width to leaf length ratio (LW/LL); 4) growth form (mat forming, rambling, caespitose); and, 5) bulk density (BD, g/cm^3)]).

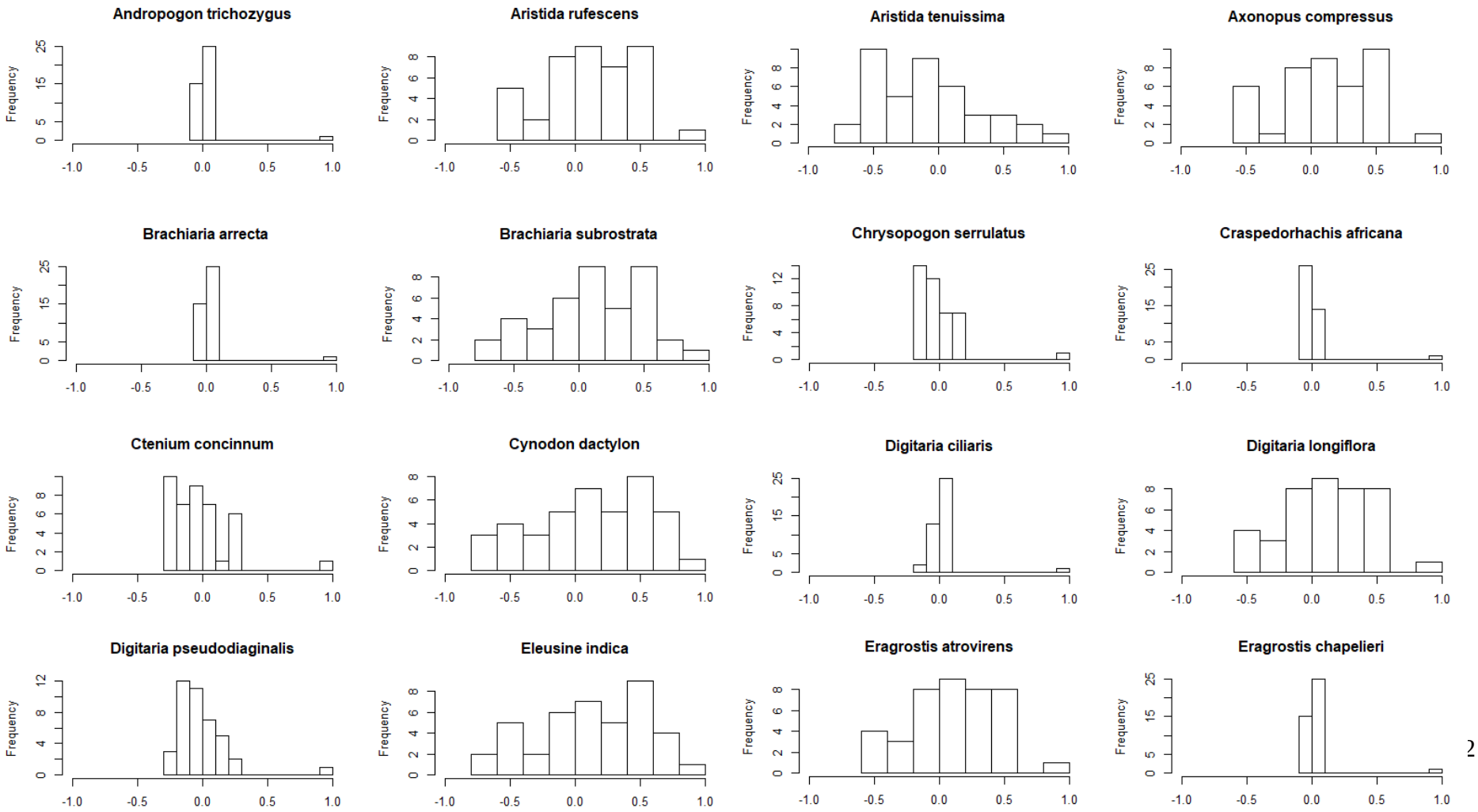
Traits	Collection method	Related function	References
Leaf table height (H_{LT}, cm)	The height visually estimated to correspond to the c. 80th quantile of leaf biomass was measured on three individuals per species.	Plant height is a key functional trait with consequences for light competition in frequently burnt environment. Tall grasses are effective competitors for light, often associated with high total biomass and are more flammable which reinforce a fire feedback to increase flammability. Tall grasses are "fire resistors and grazer avoiders". Short grasses have low proportion of stem material and are relatively higher-quality forage.	Westoby, 1998 ; Dìaz et al., 2016; D'Antonio & Vitousek, 1992; Rossiter et al., 2003; Archibald et al., 2019 ; Hempson et al., 2015

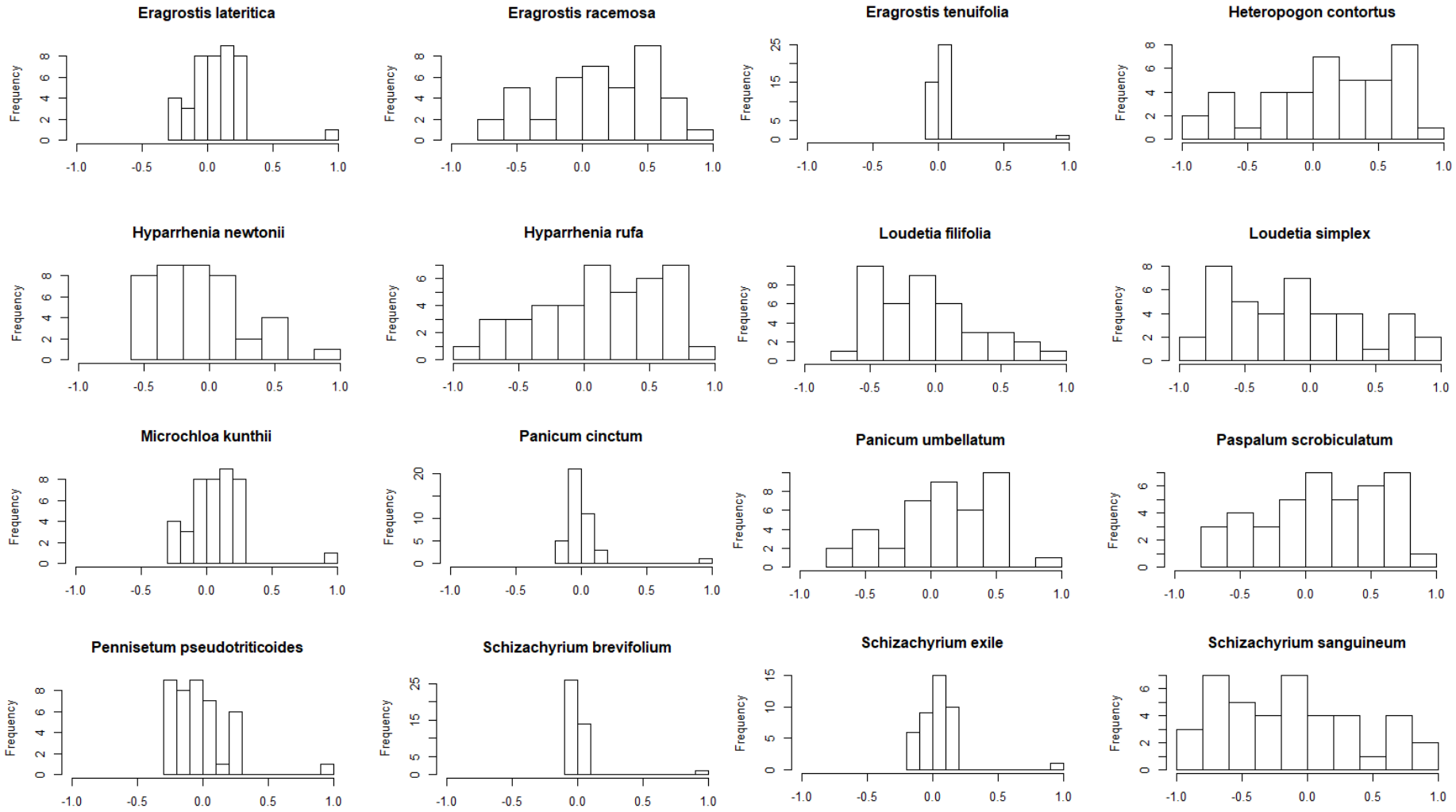
Leaf thickness (LT, cm)	Leaf thickness was measured on three fully expanded leaves on each of three individuals per species.	Leaf thickness is related to its toughness and digestibility. Toughness is among the most important mechanical attributes influencing grazing. Thick, tough leaves are less digestible to herbivores. They are hypothesized to have high carbon content to make grasses more flammable. Thinner soft leaves are more palatable and attract grazers.	Theron and Booysen, 1966; Coley, 1983; Wilson et al., 1983;
Leaf size: leaf width to leaf length ratio (LW/LL)	Leaf width and length were measured on the same three leaves per individual per species for leaf thickness measurement.	Large versus small leaves are grazing and fire attraction traits respectively. Large leaves are more palatable and preferred by grazers by reducing foraging time. Small leaves arranged in an aerated canopy ignite easily and burn intensely, i.e. more flammable.	Stobbs, 1973; Archibald et al., 2019; Schwilk, 2015
Growth form (mat forming, rambling, caespitose)	Growth form were recorded for each species.	Mat-forming habit with culms growing laterally is a grazing adaptation trait. With this growth form, most of the	Hempson et al., 2015 ; Linder et al., 2018 ; Dìaz et al.,

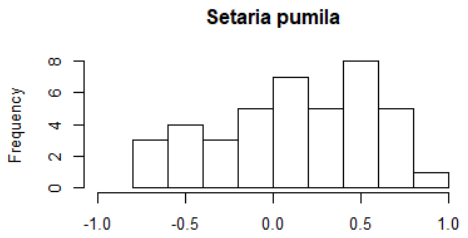
		meristematic tissues are kept below grazing depth, allowing grasses to resist intense grazing. In contrast, caespitose grasses with erect culms can protect their meristematic tissue from fire damage with intravaginal buds protected within basal leaf sheaths or underground, and tillers tightly clustered. Caespitose growth form can be associated with "generalist tolerators" and "avoiders" life histories as well. Rambling species are characterized by culms with an architecture in between prostrate and upright, which are better light competitor than mat-forming species but less than caespitose species.	2007. Hempson et al., 2019 ; Archibald et al., 2019
Bulk density (BD, g/cm^3)	Bulk density is the ratio between plant biomass and volume. It is calculated by dividing the total aboveground biomass by an estimate of the grass canopy	Species with high bulk density attract grazers with a high density of palatable leaves clustered in the canopy which promote grazing. Intermediate bulk density promotes fire spread with	Hempson et al., 2019; Coughenour, 1985.

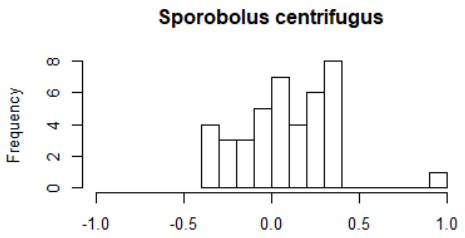
	volume. Volume was calculated using measures of the tuft basal diameter (D_B), leaf table height (H_{LT}) and leaf table diameter (D_{LT}, diameter at H_{LT}). For caespitose grasses, volume (V) was calculated using the formula for a truncated cone: $V = \pi / 3 * H_{LT} * ((D_B / 2)^2 + (D_{LT} / 2)^2 + D_B * D_{LT})$. For mat-forming grasses, a square of the individual(s) was marked out using a spade, and the volume was calculated as a cube: $V = D_B * D_{LT} * H_{LT}$. Aboveground biomass was determined on three individuals per species by clipping, drying (at 60°C for 72 h) and weighing (using a scale with two decimal place scale) the parts of the individual for which the volume estimate was made.	enough fuel to burn and sufficient air flow for combustion.	

Supplementary Figure 3: *Histograms of residual correlations values, estimated from a generalized latent variable model for each species.* Model incorporates mean annual precipitation, presence/absence of fire, distance to road and a single latent variable. Values range from -1 to +1 and species with residual correlations ranging from -0.1 to +0.1 represent a lack of any association and were not classified into assemblages.




Eragrostis lateritica
Eragrostis racemosa
Eragrostis tenuifolia
Heteropogon contortus
Hyparrhenia newtonii
Hyparrhenia rufa
Loudetia filifolia
Loudetia simplex
Microchloa kunthii
Panicum cinctum
Panicum umbellatum
Paspalum scrobiculatum
Pennisetum pseudotriticoides
Schizachyrium brevifolium
Schizachyrium exile
Schizachyrium sanguineum
Frequency


Setaria pumila
Frequency
0 2 4 6 8
-1.0 -0.5 0.0 0.5 1.0


Sporobolus centrifugus
Frequency
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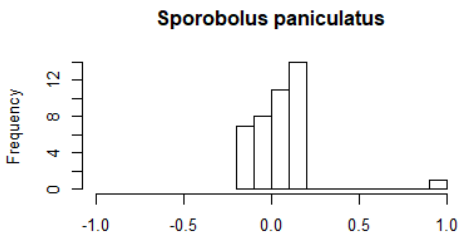
-1.0 -0.5 0.0 0.5 1.0


Sporobolus paniculatus
Frequency
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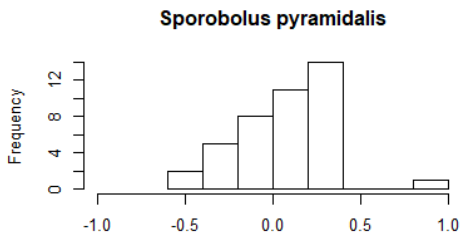
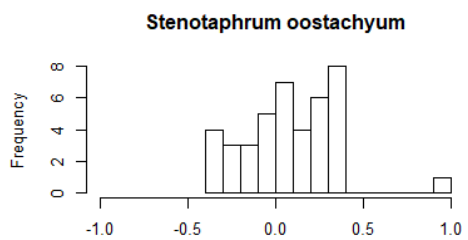
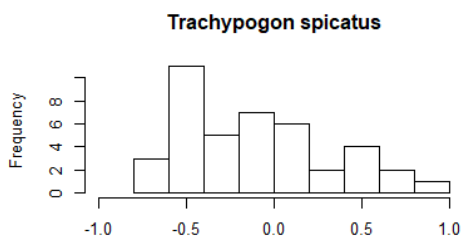
-1.0 -0.5 0.0 0.5 1.0


Sporobolus pyramidalis
Frequency
0 4 8 12
-1.0 -0.5 0.0 0.5 1.0


Stenotaphrum oostachyum
Frequency
0 2 4 6 8
-1.0 -0.5 0.0 0.5 1.0


Trachypogon spicatus
Frequency
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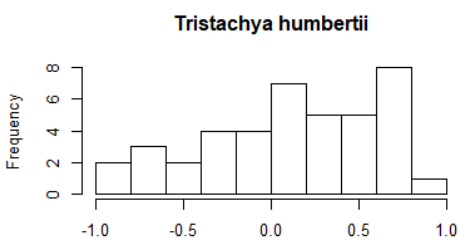
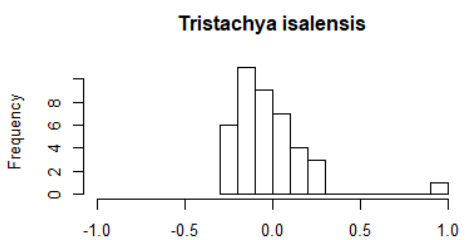
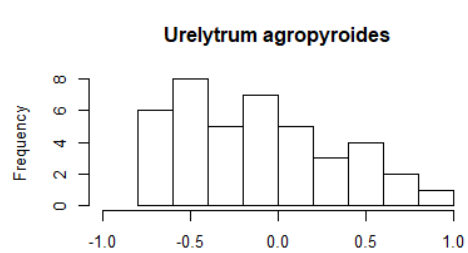

Tristachya humbertii
Frequency
0 2 4 6 8
-1.0 -0.5 0.0 0.5 1.0

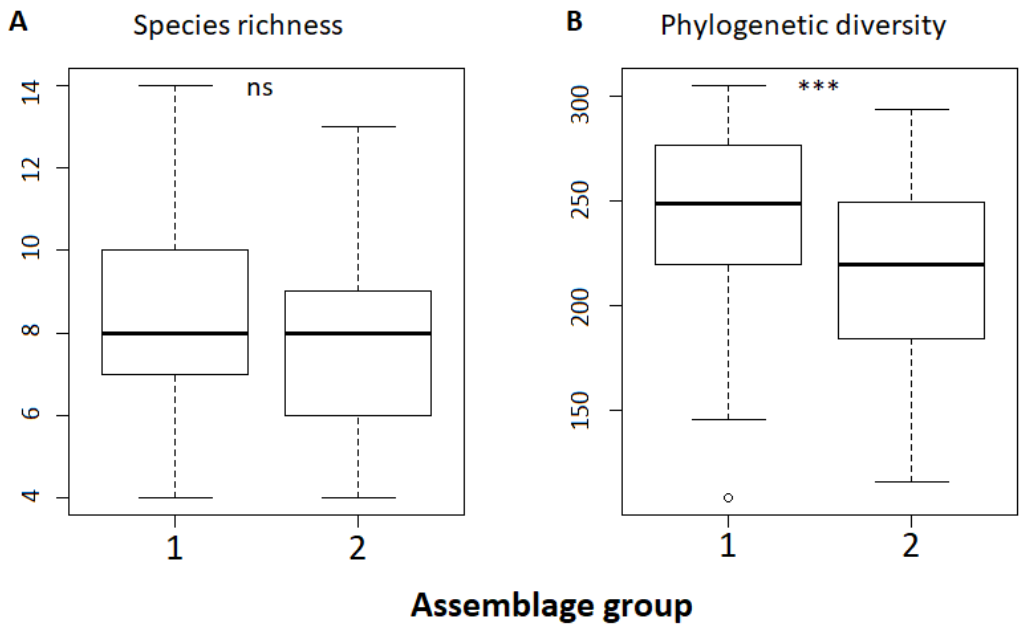

Tristachya isalensis
Frequency
0 2 4 6 8
-1.0 -0.5 0.0 0.5 1.0


Urelytrum agropyroides
Frequency
0 2 4 6 8
-1.0 -0.5 0.0 0.5 1.0

Supplementary Table 3: *Table of Akaike Information Criterion (AIC) values derived from generalized latent variable models.* Values correspond to the different environmental covariates' association used in the models of grass species frequency data in addition to a single unobserved predictor (latent variable). AIC values were sorted from the lowest to the highest and the model with mean annual precipitation (MAP), distance to road, presence/ absence of fire was kept for interpretation.

Environmental covariates used for the model	**AIC values**
MAP + distance to road + presence/ absence fire	4904.07
MAP + distance to road + presence/ absence fire + percent sand	4906.25
MAP + presence/ absence fire	4923.8
MAP + distance to road	5011.67
MAP + distance to road + percent sand	5016.9
MAP	5040.02
MAP + percent sand	5043.26
distance to road + presence/ absence fire + percent sand	5168.96
presence/ absence fire + percent sand	5179.44
distance to road + presence/ absence fire	5193.85
presence/ absence fire	5199.39
distance to road	5348.65
distance to road + percent sand	5356.67
percent sand	5363.98
null model	5393.02

Supplementary Figure 4. Grass s*pecies richness and phylogenetic diversity across assemblage group.* Assemblage groups (1 and 2) are based on residual correlations values between pairs of species as a product of the generalized linear latent variable model described in the main methods. No significant differences were found between species richness but phylogenetic diversity differed significantly between the two groups (GLM, $P < 0.001$).



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